THE INFLUENCE OF AN OXIDIZING SUBSTANCE (SODIUM IODOXYBENZOATE) ON THE CATALASE VALUE OF THE BLOOD AND TISSUES

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Sodium iodoxybenzoate is an organic peroxid. It is an iodin substitution product of orthoamido-benzoic acid containing two atoms of oxygen bound to the iodin molecule. The method of preparation of this substance and the determination of its available oxygen have been described by Loevenhart and Grove[1] and by Arkin.[2]

The pharmacology of this substance was first studied by Loevenhart and Grove[1] in the hope of obtaining some experimental evidence as to the mechanism of physiologic oxidation. Iodoxybenzoic acid and its salts seemed to be especially suited to this purpose because they can be used for intravenous injection. They found that sodium iodoxybenzoate very readily oxidizes hemoglobin to oxyhemoglobin, showing that the oxygen contained in the molecule is available for physiologic oxidation in the same manner as the oxygen taken up in the lungs. Experiments tending to show the availability of the oxygen for oxidation by the tissues were not conclusive, since sodium iodoxybenzoate was unable to furnish the oxygen necessary for the peroxid reaction. On the other hand, depression of the respiratory center and the production of long periods of apnea indicate the physiologic activity of the oxygen on the respiratory center. Following intravenous injection, there is a moderate leukocytosis which consists for the most part in an increase in the polymorphonuclear leukocytes.

The bactericidal action of sodium iodoxybenzoate has been studied by Arkin,[3] who found that it possesses a marked bactericidal action for B. typhosus, B. pyocyaneus, B. coli and Staphylococcus aureus. Moreover, its strength as a bactericide was related to its oxidizing power. In experiments with B. typhosus, sodium iodoxybenzoate proved to be 200 times as bactericidal as sodium iodbenzoate and twice as bactericidal as sodium iodosobenzoate. Sodium iodbenzoate contains no available oxygen, while sodium iodosobenzoate contains but one molecule of available oxygen or about one-half the available oxygen of iodoxybenzoate.

Sodium iodoxybenzoate in its relation to the biologic reactions has been studied by several authors. Hektoen[4] found that dogs which had been injected with goat blood followed by an intravenous injection of sodium iodoxybenzoate

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[1] Jour. Pharmacol. and Exper. Therap., 1911, 3, p. 101.
[2] Jour. Infect. Dis., 1913, 13, p. 408.
[3] Jour. Pharmacol. and Exper. Therap., 1911, 3, p. 145.
[4] Tr. Chicago Path. Soc., 1911, 8, p. 138.

produced more lysin than dogs receiving only goat blood, and suggests that the production of antibodies is related to physiologic oxidation. It is interesting to note that a single injection proved quite as effective as repeated injections. Amberg and Knox[5] have shown that the intravenous injection of iodoxybenzoate always markedly lessens the intensity of the intracutaneous reaction in rabbits sensitized with horse serum, in some cases resulting in a complete absence of reaction. This effect proved to be only temporary, lasting but a few days. These results furnish evidence that a physiologically active oxidizing agent decreases the intensity of a local inflammatory reaction. Additional evidence in support of this is the observation of Amberg[6] that the intravenous injection of sodium iodosobenzoate diminishes the inflammatory reaction produced by the subcutaneous injection of mustard oil and diphtheria toxin in rabbits.

A series of experiments to determine the effect of sodium iodoxybenzoate on the production of immune bodies and related reactions has been carried out by Arkin. Sodium iodoxybenzoate markedly increased the phagocytic activity of leukocytes in vitro,[7] and resulted in an increased production of specific hemolysin for ox corpuscles and agglutinins for the typhoid bacillus when injected into immunized rabbits.[8] It was also found to have an inhibitory effect on the local anaphylactic reaction in tuberculous guinea-pigs. These experiments show a definite relation between physiologic oxidation and the immune reactions, since a substance which contains physiologically active oxygen stimulates the production of immune bodies. Arkin has suggested that the influence of sodium iodoxybenzoate is exerted not through the liberation of the oxygen contained in the molecule, but that it stimulates the tissues which are the site of antibody formation in the manner of a catalytic agent.

Catalase is an enzyme belonging to the class of oxidases, which possesses the specific property of decomposing hydrogen peroxid with the liberation of molecular oxygen. This ferment was first isolated and named by Loew[9] in 1901. He was able to demonstrate its specificity and almost universal occurrence in plant and animal tissues. A. Schmidt[10] demonstrated that the catalase of the blood is contained almost exclusively in the red blood corpuscles, and Bergengrün[11] later proved that the stroma and not the hemoglobin was the active agent.

CATALASE OF THE BLOOD

According to Winternitz[12] the catalase value of the blood of a normal rabbit is constant from day to day, showing only such changes as come within the limits of error of the method. It differs quite markedly, however, in different individuals of the same species. Rab-

[5] Jour. Pharmacol. and Exper. Therap., 1911, 3, p. 223.
[6] Ztschr. f. d. ges. Exp. Med., 1913, 2, p. 19.
[7] Jour. Infect. Dis., 1912, 11, p. 427.
[8] Jour. Infect. Dis., 1915, 16, p. 349.
[9] Report 68, U. S. Dept. Agric., Wash., D. C., 1901.
[10] Arch. f. d. ges. Physiol., 1872, 6, p. 413.
[11] Inaug. Diss., Dorpat, 1888.
[12] Jour. Exper. Med., 1909, 11, p. 200.

bits from the same litter and similar in size and development are more nearly alike in their catalase activity than those varying in size and age. Fully developed and well nourished rabbits showed a much higher catalase activity of the blood than young and poorly developed animals. Strauss[13] reports that starvation is accompanied by a rise in catalytic activity of the blood of rabbits which returns to a lower level when feeding is resumed.

Very little work has been done to determine directly the effect of oxidation on the catalase value of the blood. Complete thyroidectomy in rabbits is followed by a marked and permanent decline in the catalytic activity of the blood, according to Winternitz and Pratt.[14] In incomplete thyroidectomy the fall, if any occurs, is only temporary. Feeding thyroid extract to thyroidectomized animals gradually raises the catalytic activity of the blood to the normal level, but never goes beyond even with excessive feeding. Thyroid extract when fed to normal rabbits did not influence their blood catalase. On the other hand Burge, Kennedy and Neill[15] report that feeding thyroid to the cat increases the catalase value of its blood and suggest that this increase in catalase of the blood may account for the increased oxidation during thyroid feeding.

Experiments.—Our experiments were undertaken as the first step in an attempt to analyze the mechanism by which sodium iodoxybenzoate, a physiologically active oxidizing agent, affects the biologic reactions. The function of catalase is still unknown, although its universal occurrence has led to the belief that it must play an important rôle in the physiologic economy of the organism. Since the only known property of catalase outside the body is its ability to decompose hydrogen peroxid, several theories have assigned to it an important rôle in physiologic oxidation. There was thus the additional possibility of obtaining some information as to the function of catalase.

Rabbits were used in pairs as nearly as possible alike in color, age and state of nutrition. The method of determining the catalase value of the blood was essentially that of Winternitz.[16] Blood was obtained from the ear and drawn into a fine pipet graduated to contain 0.025 c c. This was immediately diluted with 10 c c of distilled water, making a dilution of 1 : 400. Five c c were then placed into each of two 100 c c salt mouth bottles. A small vial containing 5 c c of hydrogen peroxid was introduced into one of the bottles which was then connected with a gas buret. After a little practice the small vial could be overturned

[13] Bull. Johns Hopkins Hosp., 1912, *23*, p. 51.
[14] Jour. Exper. Med., 1910, 12, p. 115.
[15] Am. Jour. Physiol., 1917, 43, p. 433.
[16] Arch. Int. Med., 1911, 7, p. 624.

by a single violent shake. The bottle was vigorously shaken for 1 minute to insure thorough mixing. Readings were taken after 1 minute and at intervals of 30 seconds for 3 minutes. Both samples were usually titrated and the average number of c c of oxygen liberated after 3 minutes recorded as the final result. The hydrogen peroxid used was Mallinckrodt's containing 3%. Each bottle was titrated for acidity and neutralized before use. Because of slow deterioration it was found necessary to make frequent titrations of the hydrogen peroxid by means of lead peroxid as described by Winternitz.[10]

TABLE 1

CATALASE CONTENT OF BLOOD OF RABBITS INJECTED WITH SALT SOLUTION (A) AND N/20 SODIUM IODOXYBENZOATE SOLUTION (B) (SEE CHART 1)

Date	C c of Oxygen in 3 Minutes	
	Rabbit A	Rabbit B
Before injection:		
9-16	14.8	17.2
9-17	17.2	23.0
9-18	16.0	17.0
9-19	20.2	19.8
9-22	14.5	19.0
9-23	16.3	18.7
9-24	16.1	16.9
9-25	15.9	15.6
9-27	16.9	16.8
9-28	18.1	17.4
9-29	16.5	18.1
9-30	16.1	17.1
10- 5	12.0	13.0
10- 6	10.4	10.0
10- 7	16.5	20.3
10- 9-11 a. m.	21.0	23.0
10- 9- 4 p. m.	19.5	23.7
After injection:		
10- 9- 7 p. m.	15.1	20.0
10-10	13.6	16.7
10-11	19.4	21.2
11-12	18.4	18.8
10-13	17.3	19.7
10-15	16.2	18.5
10-16	15.4	20.2
10-19	17.7	21.3
10-21	18.4	20.7
10-29	15.6	16.3
11- 6	17.5	20.4
11-16	19.2	22.6
11-21	19.5	20.8

Table 1 contains the data on the daily variation of the catalase activity of a pair of rabbits before and after injection. Rabbit A served as control and was injected with 5 c c of sterile salt solution, while Rabbit B was injected with 5 c c of neutral N/20 sodium iodoxybenzoate solution. The control rabbit showed a daily variation ranging from 10.4 c c, the lowest, to 21 c c, the highest, or a maximum difference of 10.6 c c. Following the injection of salt solution the greatest variation was 5.9 c c. The catalase value of the blood of Rabbit B before injection varied from 10 c c-23.7 c c, giving a difference of 13.7 c c

under normal physiologic conditions. Two and one-half hours after the injection of sodium iodoxybenzoate there was a fall of 3.7 c c which increased within 24 hours to 7 c c. These differences lie well within the range of normal physiologic variation. When plotted graphically (Chart 1) the curves of catalase variation in the two animals before and after injection show no marked differences. Several series of animals were treated in the same way over a shorter period of time with the same results. Other experiments gave the same results.

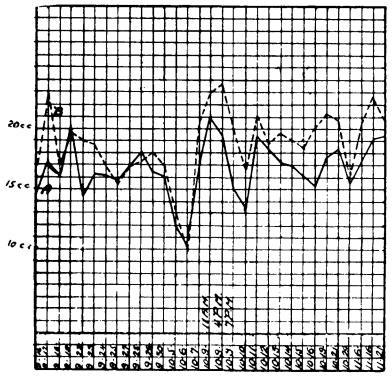

Chart 1.—Showing the daily variation in catalase content of the blood before and after injection. Both animals injected 4:30 p. m., 10/9/14 (note Table 1).

CATALASE OF TISSUES

Battelli and Haliff[17] in a study of the catalase value of the tissues of various species of animals found that the tissues of an animal show quite marked differences in catalytic activity. The same tissue from animals of different species shows a difference in activity, but within a given species the same organ possesses a constant catalase value among the individuals of that species. The catalytic activity was not in proportion to body temperature, since cold blooded animals such as the adder have tissues richer in catalase than many warm blooded animals such as the rabbit. In a general way the catalytic value of the tissues of the various animals studied in the order of decreasing activity was as follows: liver, kidney, blood, spleen, lung, heart, muscle, and brain. In most species the liver possesses the greatest catalytic activity. The kidney of the rabbit is slightly richer in catalase than the liver.

[17] Compt. rend. Soc. de biol., 1904, 2, p. 264.

Spitzer,[18] by comparing the power of tissues to decompose hydrogen peroxid with their power to oxidize salicylic aldehyd noted a similarity in the two cases, and concluded that the power of tissues to decompose hydrogen peroxid is a true measure of their oxidizing power. Spitzer's work has been questioned by Battelli and Stern[19] who point out that his methods were faulty and not based on a quantitative determination of active catalase. Moreover, he assumed that the substance which actively decomposes hydrogen peroxid is identical with the other oxidases, and that this reaction is of the same type as all fermentative oxidations. Subsequent work has demonstrated that catalase is a specific enzyme. Kastle and Loevenhart[20] found that HCN strongly inhibits the action of liver catalase on hydrogen peroxid, thus showing that a substance which interferes with physiologic oxidation also interferes with the action of catalase. Using corresponding muscles for comparison, Burge[21] found a greater catalytic activity in warm blooded animals in which oxidation is more intense than in cold blooded animals. The amount of catalase in the different muscles of the body varies with the amount of work which they are normally called on to perform, and in any single muscle increases with an increase in external physical work. It would seem, therefore, that the catalase content of a muscle is directly proportional to the amount of oxidation in that muscle.

Experiments.—Since we were unable to demonstrate any change in the catalase value of the blood of rabbits following the intravenous injection of sodium iodoxybenzoate, our next problem was to determine its effect on the catalase value of the tissues.

Here again, the method is essentially that of Winternitz.[22] Rabbits were used in all experiments. The organs were rendered as nearly blood free as was possible without washing them, by bleeding the animals from both carotids. Ten gm. of the fresh organ were ground in a mortar with washed white sand. To this was gradually added 20-30 c c of distilled water and the mixture strained through clean cloth. The residue was ground again, distilled water added, and strained as before. This was repeated once more and the combined filtrates diluted with distilled water to 100 c c, making a 10% aqueous extract. In the case of organs weighing less than 10 gm., the whole was ground up with enough water to make a 10% emulsion. One c c of the emulsion to be tested was diluted to 5 c c with distilled water in a 100 c c salt mouth bottle, hydrogen peroxid added and the catalase value determined as previously described. In our preliminary experiments we used 5 c c of neutralized hydrogen peroxid, but later

[18] Quoted by Kastle and Loevenhart.[20]
[19] Arch. di Fisiol., 1905, 2, p. 471.
[20] Am. Chem. Jour., 1903, 29, p. 397.
[21] Am. Jour. Physiol., 1916, 41, p. 153.
[22] Jour. Exper. Med., 1908, 10, p. 759.

experience showed this to be insufficient for the organs rich in catalase. In the experiments reported 10 c c of neutralized Mallinckrodt's hydrogen peroxid were used. We also found it necessary to standardize our hydrogen peroxid against lead peroxid each time before use. Duplicate determinations were always made and the tabulated results represent the average number of c c of oxygen liberated by the action of 0.2 c c of a 10% emulsion on 10 c c of 3% hydrogen peroxid in 3 minutes.

Table 2 contains the results of the determinations of the catalase value of the kidney, liver, spleen, lungs and muscle for six normal rabbits in a decreasing order of activity. We find, as did Battelli and Haliff, that in the rabbit the kidney shows the highest catalytic activity,

TABLE 2

CATALASE VALUE OF ORGANS OF NORMAL RABBITS*

Kidney	Liver	Spleen	Lungs	Muscle
105.5	110.4	68.8	30.8	2.4
105.3	100.5	35.0	20.2	1.9
95.0	85.6	27.8	25.6	2.4
104.5	81.9	18.0	23.3	2.4
70.0	67.9	26.3	16.8	
83.6	85.3	35.3	22.5	

*C c of oxygen in 3 minutes, using 0.2 c c of 10% emulsion and 10 c c of neutral hydrogen peroxid.

TABLE 3

CATALASE VALUE OF ORGANS OF RABBITS INJECTED WITH SODIUM IODOXYBENZOATE*

	Kidney	Liver	Spleen	Lungs	Muscle
Two animals killed 1 hour after injection	104.5 99.5	76.2 86.2	42.8 67.4	41.2 31.7	1.8 2.4
Two animals killed 24 hours after injection	106.0 99.3	111.0 73.6	50.8 51.9	40.2 21.4	3.0 2.4
Two animals killed 10 days after injection	103.0 98.6	76.4 99.5	42.3 32.5	19.8 26.7	2.2 2.0

*C c of oxygen in 3 minutes using 0.2 c c of 10% emulsion and 10 c c of neutral hydrogen peroxid.

being slightly more active than the liver except in one animal. Of all the tissues tested, skeletal muscle was the lowest in activity and the most constant. The catalase values of the tissues of the individual animals show considerable differences, but we have noted that the more nearly the animals are alike as to color, size and general state of nutrition the more closely does the catalase value of their tissues agree.

Table 3 shows the catalase value of the tissues of rabbits injected with 5 c c of neutral N/20 sodium iodoxybenzoate solution intra-

venously, and killed at varying intervals following injection as indicated in the table. None of the organs shows values markedly differing from those of the normal animals, which might not be accounted for as differences between individuals of the same species. The spleen has a rather high value in the injected animals, but no definite conclusions can be based on this because of the wide variations among normal animals.

CONCLUSIONS

The catalase value of the blood of normal rabbits may show considerable daily variation under normal conditions, and is not as constant as previous experiments would seem to indicate. Any change in catalase content due to experimental conditions must therefore be excessively high or excessively low before it can be attributed to such conditions.

The intravenous injection of sodium iodoxybenzoate in normal rabbits has no effect on the catalase value of the blood, that might not be accounted for as a normal physiologic variation.

We have found rather marked variations in the catalase activity of tissues of normal rabbits.

Rabbits injected intravenously with sodium iodoxybenzoate do not show catalase values of their organs markedly higher or lower than those occurring among normal rabbits. A possible exception is the spleen which shows an increase in catalytic activity. However, this increase is not greater than might be accounted for on the basis of individual variation.